ABSTRACT

IMPROVED CIRCULAR SITE-DIRECTED MUTAGENESIS

The invention provides improved methods of introducing site-directed mutations into circular DNA molecules of interest by means of mutagenic primer pairs. The mutagenic primer pairs are also selected so as to be either completely complementary or partially complementary to each other, wherein the mutation site (or sites) is located within the region of complementarity. A mutagenic primer pair is annealed to opposite strands of a circular DNA molecule containing the DNA sequence to be mutagenized. After annealing, first and second mutagenized DNA strands, each incorporating a member of the mutagenic oligonucleotide primer pair is synthesized by a linear cyclic amplification reaction. After the linear cyclic amplification mediated synthesis step is completed, the reaction mixture is treated with a selection enzyme that digests the parental template strands. After the digesting step, a double-stranded circular DNA intermediate is formed. The double-stranded circular DNA intermediates is transformed in suitable competent host cells and closed circular double- stranded DNA corresponding to the parental template molecules, but containing the desired mutation or mutations of interest, may be conveniently recovered from the transformed cells. The invention also provide kits for site-directed mutagenesis in accordance with methods of the present invention.